The significance of peroxisome function in chronological aging of Saccharomyces cerevisiae

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Supporting information

Supplementary tables

Table S1: Yeast strains used in this paper.

Strains	Description	Reference
WT	BY4742 MATα *his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0*	
Δ*pex3*	BY4742 Δ*pex3::KanMX4*	Euroscarf collection
Δ*pex5*	BY4742 Δ*pex5::KanMX4*	Euroscarf collection
Δ*pex6*	BY4742 Δ*pex6::KanMX4*	Euroscarf collection
Δ*3'pex5*	BY4742 Δ*3'pex5::KanMX4*	this study
Δ*pot1*	BY4742 Δ*pot1::KanMX4*	Euroscarf collection
Δ*tgl3*	BY4742 Δ*tgl3::KanMX4*	Euroscarf collection
Δ*pex3*Δ*pot1*	BY4742 Δ*pex3::KanMX4* Δ*pot1::NatMX4*	this study
Δ*pex7*	BY4742 Δ*pex7::KanMX4*	Euroscarf collection
WT GFP.SKL	BY4742 *pMET25-GFP.SKL/Zeo*^{R}	this study
Δ*atg1*	BY4741 Δ*atg1::KanMX4*	Euroscarf collection
Δ*atg1*Δ*pex3*	BY4741 Δ*pex3::KanMX4* Δ*atg1::NatMX4*	this study
Δ*pex5/PEX5*	BY4742 Δ*pex5::KanMX4* pRS316-*PEX5*	this study

Table S2: Primers used in this paper.

Primers	**Sequence 5'-3'**
Pex5UP	TATGCAAAGGTTCATAAACGGAGAACCACTGATCGATGATAAAAGAAGA A-CAGCTGAAGCTTCGTACGC
Pex5DN	CTCTCTTCAAAGTCTCTATAACAGTATCATTGTACGTATTCAAGAGAGAT-GCATAGGCCACTAGTGGATCTG
Pex5.1	GGCGTCTTAATGAGTCACCT
Pex5.2	ATGCCTGGCTTCACTTCTTG
Pex5.5	ATCCGCTCAGAGTATCTTCG
Pex5.6	TCCATGTCTCTTCGCATAGG
Pex5.A	GCTTGCTGATTTTACCTGATGTATT
Pex5.B	GAGAGCTTTTCTCTCCCTGATAAAC
Pot1.1	CTACAGCTGCTAACGCTACACCGACCAA
Pot1.2	CTAGGATCCCTGTACTCAGAGCCACAAG
Pot1.3	CTAACTAGTGCCGCCGCCATCTT
Pot1.4	CTACCGCGGACGTTACCTCATATGGCTATCG
Pot1.5	GAGGCATGCACTTCGGATTA
Pot1.6	AATTCAACGCGTCTGTGAGG
Pot1.7	GACATCATCTGCCCAGATGC
Pot1.8	TGGAGGGGAAGAAGTGAGAG
GFPSKL-3	TATCCGCGGCGCGCAATTAACCCTCA
GFPSKL-4	TATGCGGCCGCGTAACGCCAGGGTTTT
MET25.1	GGCGTCAGATTTAGGTGGAT
ATG1up	TTCAAATCTCTTTTACAACACCAGACGAGAAATTAAGAAA-GACGGATCCCCGGGTTAATTA
ATG1down	GGTCATTTGTACTTAATAAGAAAACCATATTATGCATCAC-CGACACTGGATGGCGGCGTTA
Atg1.1	CTGGGGAAACAGAGAACAGT

Supplementary experimental procedures

Construction of Δ*atg1*Δ*pex3* strains

ATG1 gene was deleted in Δ*pex3* cells by replacing the open reading frame with nourseothricin resistance gene {Goldstein, 1999 #32}. The *atg1::NatMX4* DNA fragment was amplified with ATG1up and ATG1down primers (Table S2) using pAG25 {Goldstein, 1999 #32} as template and transformed into Δ*pex3* cells. Correct insertion by homologous recombination was confirmed by colony PCR using Atg1.1 and Pot1.6 primers (Table S2).

Chronological aging experiment on peroxisome induction medium for *pex* mutants

Overnight cultures were grown in MM medium containing 0.5% glucose and required amino acids. Those cultures were then diluted twice at $OD_{600\ nm}$ = 0.1 in fresh MM containing 0.3% glucose and grown for 8 hours. After the last pre-cultivation step, cells were diluted in MM containing 0.25% ammonium sulfate, 0.05% yeast extract, 0.1% oleic acid and 0.05% Tween 80 and 0.1% glucose. Cultures were incubated at 30°C, 200 rpm. Survival was assayed by counting colony-forming units (CFUs) after 2 days of incubation at 30°C on YPD agar plates. 24 hours after the last dilution (D1) was considered as 100% of survival. The results shown are mean values and standard error of mean. Statistical analyses were determined using two-way ANOVA. A p value of less than 0.05 was considered as a significant difference.